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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/607,903	06/27/2003	Gjalt W. Huisman	MBX 025 DIV CON	7536

23579 7590 06/22/2010

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EXAMINER	
HUTSON, RICHARD G	
ART UNIT	**PAPER NUMBER**
1652	
MAIL DATE	**DELIVERY MODE**
06/22/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	**Application No.** 10/607,903	**Applicant(s)** HUISMAN ET AL.	
	Examiner Richard G. Hutson	**Art Unit** 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 May 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,6,7,11,12,14-16,19 and 21* is/are pending in the application.

4a) Of the above claim(s) *11,12,14-16,19 and 21* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,6,7* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/3/2010 has been entered.

Applicant's cancellation of claim 2, 8 and 21 and amendment of claims 1, 3, 7, 11, 12, 19, in the paper of 5/3/2010, is acknowledged. Claims 1, 3, 4, 6, 7, 11-12, 14-16, 19 and 21 are still at issue and are present for examination.

Applicants' arguments filed on 5/3/2010, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 11, 12, 14-16, 19 and 21 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 102

The rejection of claims 1, 2, 4, 6 and 8 under 35 U.S.C. 102(b) as being anticipated by Liebl et al. (J. Bacteriology 174(6): 1854-1861 (1992)), is hereby with drawn based upon the cancellation of claims 2 and 8 and applicants amendment of

claims 1, 4 and 6 requiring that the claimed bacterial strain produce polyhydroxyalkanoates. The bacterial strain taught by Liebl et al. does not produce polyhydroxyalkanoates.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 4, 6 and 7, are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

A similar rejection was stated in the previous office action as it applied to previous claims 1-4 and 6-8. In response to this rejection applicants have cancelled claims 2, 8 and amendment of claims 1, 3, 7, and traverse the rejection as it applies to the claims in light of the filed declaration.

The rejection as it applies to applicants newly amended claims is repeated herein. Greer et al. teach that the degradation or removal of nucleic acids from cell

lysates during fermentation is important because they form solutions of high viscosity which interfere with subsequent processing. Greer et al. specifically teach the usefulness of peroxide degradation in the recovery of intracellularly produced materials, in particular polyhydroxyalkanoate polymers, from bacterial cell lysates. Greer et al. further teach that nucleases can also be added to a cell lysate in order to degrade the nucleic acid although nucleases are expensive (page 1, lines 25-31).

Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Corynebacterium glutamicum is closely related to other “amino acid-producing corynebacteria” and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Miller et al. teach the secretion and processing of *Staphylococcal aureus* nuclease in *Bacillus subtilis*.

Atkinson et al. teach all aspects of biochemical engineering and biotechnology, including properties of microorganisms, microbial activity, product formation, fermentation processes, downstream processes and product recovery processes. Atkinson et al. also teach many products that can be produced biochemically such as antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides. Atkinson et al. specifically teach many of the industrial production

characteristics for a number of commercially important compounds, for example Atkinson et al. teach that *Alcaligenes eutrophus (Ralsotnia eutropha*) has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32).

Lee et al. teach several processes developed for the production of various poly(hydroxyalkanoic acids) including various microorganisms used and the optimization of fermentation conditions.

One of ordinary skill in the art would have been motivated to genetically engineer a *Alcaligenes eutrophus (Ralsotnia eutropha*) polyhydroxyalkanoates producing bacterial strain, as taught by Atkinson et al., to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would express a nuclease which is secreted into the periplasmic space in an amount effective to degrade at least 95% of all the nucleic acid released follosing lysis of the cells in less than 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of product is enhanced. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would a inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the

viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous heterlogous nuclease gene into the chromosome of the bacterial host so that the nuclease activity can be increased for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, polyhydroxyalkanoates as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans*, *Pseudomonas acidovorans* and *Escherichia coli* or any

other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that *Alcaligenes eutrophus* (Ralstonia eutropha) has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

Applicants Argument:

As in previous responses to similar rejections, applicants again review the legal standard, and then review applicant's interpretation of what each of the references teaches. After this analysis, applicants submit that "A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims". In response to this argument applicants are again reminded that this rejection is based upon the obviousness of the claims in light of the teachings of the prior art references and that it is unnecessary for the combination of Greer, Liebl, Miller, Atkinson and Lee to recite all of the elements of the claims, in order for the claims to render obvious the rejected claims.

Further, applicants are again reminded that applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re*

Keller, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

As in previous responses to similar a similar 103 rejection based upon these same references, applicants again submit that none of the references cited by the Examiner disclose or suggest the claimed bacterial strain. Applicants submit that specifically none of the references cited by the Examiner disclose expression and secretion of a nuclease into the periplasmic space of any gram negative bacteria, in any amount (see Liebel declaration filed December 10, 2009), nor production of PHAs.

Applicants again submit that none of the references disclose the problem to be solved except for Greer, which teaches away from the claimed solution to this long standing problem, by advising those skilled in the art to add exogenous nuclease to the lysate. Applicants submit that they provide an elegant system for reducing viscosity in fermentation media whereby bacteria are engineered to express an effective amount of nuclease, which is sequestered in the periplasmic space where it is harmless to the cells, until it is released when needed. There is nothing in any of the references cited by the Examiner which would lead one of ordinary skill to this limitation.

Applicants submit that Liebl does not disclose expression of the SNase gene product in *E. coli, as* Liebl discloses propagation of a plasmid containing the SNase gene. Thus if the *E. coli* in Liebl were lysed, the plasmid would be released, not the nuclease. There is no basis for the assertion that cell lysis will lead to secretion of the gene product into the periplasmic space. With respect to the C. *glutamicum* disclosed in

Liebl, is it unclear how the bacteria which are engineered to secrete nuclease into cell culture medium would secrete the nuclease into the periplasmic space if lysed. *C gtutamicum* does not have a periplasmic space. *See* Zuber, *J. Bacteriol.,* 190(16):5672-5680 (2008) (a copy of which was previously submitted December 10, 2009). See especially, Fig. Legend of Fig. 1).

Applicants submit that none of Atkinson or Lee makes up for the deficiencies in Greer, Liebl and Miller, as Lee discloses the production of copolyesters (not PHAs) in *Pseudomonas sp.* Lee does not disclose genetically modifying any bacteria for secretion of nuclease into the periplasmic space

Applicants submit that Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency.

Applicants complete amendment of the claims and applicants above argument is acknowledged and has been carefully considered, however, continues to be found nonpersuasive. As previously stated to applicants, applicants are again reminded that applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants are reminded that this rejection is based upon obviousness of all of the references of the rejection combined in light of the knowledge at the time of invention. As stated above, however, not specifically addressed in applicants response,

one of ordinary skill in the art would have been motivated to genetically engineer a *Alcaligenes eutrophus (Ralsotnia eutropha*) polyhydroxyalkanoates producing bacterial strain, as taught by Atkinson et al., to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would express a nuclease which is secreted into the periplasmic space in an amount effective to degrade at least 95% of all the nucleic acid released follosing lysis of the cells in less than 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of product is enhanced. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. It is noted that The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. Applicants comments that Greer actually teach away from the invention, because they do not genetically engineer a bacteria as such is noted but not found persuasive. Greer provide the motivation to solve the

problem, however, they chose to solve it by a different means. The combination of the references would make it obvious to solve the problem through genetic engineering. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous heterlogous nuclease gene into the chromosome of the bacterial host so that the nuclease activity can be increased for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, polyhydroxyalkanoates as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Alcaligenes eutrophus*, *or Escherichia coli* or any other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that *Alcaligenes eutrophus* (Ralstonia eutropha) has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass,

page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

Applicants regarding the teaching of Liebl et al. are acknowledged, however, not considered relevant to the current rejection, in which it is obvious that the genetically engineered bacterial strain be *Alcaligenes eutrophus* (Ralstonia eutropha), not C. glutamicum.

In addition to the above, applicants argue specifically with respect to claim 7, that none of the prior discloses genetically modifying bacteria with the heterologous nuclease gene integrated into the chromosome, and the gene product secreted into the periplasmic space.

Applicants argument with respect to the periplasmic space has been addressed previously and above, as any nuclease expressed would move through the periplasmic space upon lysis of the cell. With respect to the integration of the heterologous nuclease gene into bacterial chromosome, this is but a common means of transformation of a bacteria.

Finally submit *Evidence of secondary considerations.*

Applicants submit that the claims provide a simple yet elegant mechanism to endogenously produce nuclease in genetically modified bacteria, which enables cost effective processing of nucleic acids released during fermentation processes, avoids any deleterious effects of the expressed nuclease on the cells, and provides external control of the release of the nuclease. Applicants submit that this is accomplished by

directing expression of the nuclease to the periplasmic space. Applicants submit that such expression sequesters the nuclease, protecting the cells from the nuclease until needed, and enables release of nuclease into the fermentation mediums when desired. Applicants submit that none of the references cited by the Examiner, alone or in combination provide bacteria with the properties of the claimed bacteria.

Applicants argued secondary considerations have been considered and are not persuasive in overcoming the current rejection. While applicants may feel that they have provided a simple yet elegant mechanism to endogenously produce nuclease in genetically modified bacteria, it remains that the claims as they currently read remain obvious over the teachings of the prior art for the reasons stated above.

While the claims and applicants specification may provide microbial strains that are cost effective for fermentation processes and can enable more profitable production of polyhydroxyalkaaoates, it remains that applicants claims are obvious over the reverences cited for the reasons presented above.

Thus claims 1, 3, 4, 6 and 7, are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

rgh
6/19/2010

/Richard G Hutson/
Primary Examiner, Art Unit 1652